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Pancreatic Cancer and Drinking Water and Dietary Sources of Nitrate and Nitrite

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N-Nitroso compounds, known animal carcinogens, are formed endogenously from drinking water and dietary sources of nitrate and nitrite. The authors conducted a population-based case-control study of pancreatic cancer in Iowa to determine whether increased consumption of nitrate and nitrite from drinking water and dietary sources was associated with risk. They linked detailed water source histories to nitrate measurements for Iowa community water supplies. After exclusions for insufficient data, 1,244 controls and 189 pancreatic cancer cases were available for analysis. Among controls, the median average nitrate level (1960–1987) was 1.27 (interquartile range, 0.6–2.8) mg of nitrate nitrogen per liter of water. No association was observed between pancreatic cancer risk and increasing quartiles of the community water supplies' nitrate level. Increasing intake of dietary nitrite from animal sources was associated with an elevated risk of pancreatic cancer among men and women (highest quartile odds ratios = 2.3, 95% confidence interval: 1.1, 5.1, for men and 3.2, 95% confidence interval: 1.6, 6.4, for women). In contrast, dietary nitrate intake showed an inverse association with risk among women and no association among men. This study suggests that long-term exposure to drinking water nitrate at levels below the maximum contaminant level of nitrate nitrogen (10 mg/liter) is not associated with pancreatic cancer; however, the consumption of dietary nitrite from animal products may increase risk.

diet; nitrates; nitrites; pancreatic neoplasms; water; water pollution

Abbreviations: CI, confidence interval; OR, odds ratio.

Pancreatic cancer is the fourth leading cause of cancer death among both men and women in the United States (1). Smoking is the only established risk factor. Chronic pancreatitis and a family history of pancreatic cancer are predisposing factors but both are rare. High consumption of meat, animal protein, and fat has been associated with increased risk, whereas fruits and vegetables have often been linked to a reduced risk (2). Despite continuing efforts, the etiology of pancreatic cancer is poorly understood (2–5).

Animal studies provide strong evidence for the carcinogenicity of *N*-nitroso compounds. *N*-Nitroso compounds have been shown to cause tumors in every animal species tested and to induce cancer in many different organs including the pancreas (6, 7). Specific *N*-nitroso compounds cause pancreatic cancer in hamsters, which have been used extensively as animal models for evaluating potential pancreatic carcinogens because of the morphologic and clinical similarities with pancreatic tumors in humans (7).

Although the evidence is more equivocal for humans, several *N*-nitroso compounds are classified as reasonably anticipated to be human carcinogens by the National Toxicology Program (8) and are classified as possible or probable human carcinogens by the International Agency for Research on Cancer (9). Several of these *N*-nitroso compounds can be formed endogenously (in vivo) from dietary amine precursors and nitrite (9). *N*-Nitroso compound formation in healthy individuals occurs primarily in the stomach through reaction of nitrite with amine and amide precursors. Nitrite is predominantly derived from ingested nitrate, although preserved meats and some other foods can serve as direct sources. Vegetables are the major source of nitrate exposure when drinking water levels are

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low; however, drinking water contributes substantially to exposure when nitrate levels are near or exceed the US Environmental Protection Agency's maximum contaminant level of 10 mg of nitrate nitrogen (NO_3-N) per liter of water. An increased endogenous formation of *N*-nitroso compounds was demonstrated among Nebraskan men drinking water from private wells with increasing levels of nitrate (10).

Nitrate in drinking water has long been considered a health threat for its ability to induce methemoglobinemia, and this health outcome is the basis of the Environmental Protection Agency's maximum contaminant level (11). Groundwater underlying agricultural areas often has elevated nitrate levels due to agricultural runoff of nitrogen fertilizers. Intake of nitrate from drinking water and dietary sources may cause increased exposure to *N*-nitroso compounds through endogenous nitrosation (10, 12, 13). Vegetables are the primary dietary source of nitrate; however, they contain vitamin C and other nitrosation inhibitors (14) and, therefore, high intakes may not result in high rates of formation of *N*-nitroso compounds. We hypothesized that individuals with higher daily nitrate intake from drinking water and lower intakes of nitrosation inhibitors may be at elevated risk of pancreatic cancer.

In spite of the biologic plausibility for a role of drinking water and dietary sources of nitrate and nitrite in cancer risk, few epidemiologic studies with historical exposure data have been conducted. Most of the epidemiologic studies have focused on gastric cancer (15). Whereas several studies evaluated dietary sources of nitrite and pancreatic cancer risk, only one study in Iowa (16) evaluated drinking water nitrate levels.

We conducted a population-based, case-control study of pancreatic cancer in Iowa. The study was originally designed to evaluate the possible association between disinfection byproducts and six cancer sites (17, 18). The detailed information on historical water sources, tap water intake, and diet allowed us to conduct additional analyses to evaluate the relations between pancreatic cancer and nitrate levels in drinking water and nitrite intakes from the diet.

MATERIALS AND METHODS

Study population

The overall study population was described previously (17, 18). Eligible cases were White residents of Iowa who were aged 40–85 years, diagnosed with pancreatic cancer during the years 1985–1987, and without previous diagnosis of a malignant neoplasm. Microscopic confirmation was an eligibility criterion for cases in this study. All cases were confirmed by either positive histology (n = 348) or cytology (n = 28) as documented by Registry staff through medical record review. Other races were excluded because of small numbers. Cases were ascertained through the Iowa Cancer Registry, a statewide cancer registry that is one of the National Cancer Institute's Surveillance Epidemiology and End Results Program registries.

Controls were frequency matched to cases of all six cancer types combined by gender, race, and 5-year age groups, resulting in an approximate 6.5:1 matching ratio for the pancreatic cancer cases. Controls aged less than 65 years were selected from computerized state drivers' license records, and controls aged 65 years or more were selected from a randomized listing provided by the US Health Care Financing Administration, which is now known as the Centers for Medicare and Medicaid Services. Individuals with previous cancer diagnoses were excluded.

Interviews

The study was approved by an institutional review board at the University of Iowa. A letter was sent to study subjects explaining the study and inviting participation through the completion of a postal questionnaire. To minimize refusals, reluctant participants were offered an abbreviated telephone interview as an alternative to the postal survey. Interviews were conducted by trained interviewers who were employees of the University of Iowa. Missing information for specific questions in the returned postal survey was retrieved by follow-up telephone contact.

The postal questionnaire contained questions about dietary habits, smoking history, and a complete residence and drinking water source history. In the residential history section, participants were asked to list all towns or cities in which they lived for 1 year or more from the time of birth, as well as their primary water source in each location (private well, community supply, bottled water, or other type of supply). We also asked about tap water intake and beverages made with tap water from water sources at home and away from home. Residential water sources accounted for the large majority of tap water intake (19). Our data indicated that drinking water consumed away from the residence was usually from the same community water supply (i.e., the same town), so we considered total tap water intake in our analysis.

Of 429 eligible pancreatic cancer cases, 376 (87.6 percent) participated by completing the mailed survey (n = 343) or the abbreviated telephone interview (n = 33). Proxy or self-questionnaires were mailed on the basis of the information from the initial telephone contact. Of the 376 respondents, 322 (86 percent) requested proxy questionnaires. Of the 54 direct questionnaires, 31 were completed by the cases themselves and 17 were completed by a proxy; six did not report the respondent, resulting in 339 (90.2 percent) proxies, 31 (9.1 percent) direct respondents, and six (1.6 percent) unknown.

Of 999 eligible controls aged less than 65 years, 817 (81.8 percent) participated. Of the 2,034 eligible controls aged 65 years or more, 1,617 (79.5 percent) participated, with 168 (8 percent) by abbreviated telephone interview. There were a total of 2,064 (84.8 percent) direct respondents and 243 (10.0 percent) proxies, including two sent proxy questionnaires and 241 sent direct questionnaires who indicated completion by a proxy, and 127 (5.2 percent) were unknown.

Drinking water nitrate exposure assessment

Details of the exposure assessment for nitrate levels in community water supplies were published previously (19). Briefly, an extensive water quality database containing

historical monitoring data for Iowa community water supplies was available through Iowa's Center for the Health Effects of Environmental Contamination. The earliest available nitrate measurements for some communities were made in 1934. Monitoring data were sparse before the 1970s. In the 1980s with the full implementation of the Clean Water Act, nitrate-monitoring data were available for all community water supplies, defined as suppliers serving populations of 25 or more.

To compute our exposure estimates, we used nitrate measurement data for finished water samples taken in the water distribution system after treatment. A finished water sample should be more representative of the water quality distributed to households than pretreated water or water quality data for individual community water supplies' sources. When more than one finished water nitrate level was available for a given year, an average was computed. Towns that purchased water were assigned the water quality data from the host community water supplies.

We imputed annual nitrate values for years when a town was missing nitrate data by computing a weighted average of the annual nitrate means in neighboring years. Weights were based on the number of years between the missing year and the year with measurements. The actual measurement could be before or after the missing year as long as the source did not change during the period with missing data. The weights were 1, 0.75, 0.5, and 0.2 for 1–2 years, 3–4 years, 5–6 years, and 7–9 years, respectively. When there were no data within 10 years, the nitrate level was considered to be unknown.

Yearly nitrate means (actual and imputed data) were linked to individual residential water source histories by town and year. Nitrate levels were unknown during periods when a person lived outside Iowa, resided in a town during a period with no nitrate data within 10 or more years, or drank water from a private well. Private wells can have substantially higher nitrate levels than the community water supplies, but no historical monitoring data were available. We excluded individuals from our analysis if their drinking water nitrate levels were unknown for more than 30 percent of the period after 1960 (187 cases, 1,190 controls). This decision was based on balancing the completeness of the exposure data with the available sample size.

The average nitrate exposure level from 1960 onward was calculated for each case and control. Exposure was calculated to the year of diagnosis for cases and to 1987 (the last year of case diagnosis) for controls. We also computed the number of years from 1934 onward that an individual was exposed to community water supplies with a yearly nitrate estimate at or above 7.5 and 10 mg of nitrate nitrogen per liter (the Environmental Protection Agency maximum contaminant level).

Dietary nitrate and nitrite

Dietary intakes were assessed using a 55-item food frequency questionnaire. Participants were asked to report their usual adult intake after excluding any changes to their diet in the last few years. Dietary intakes of nitrate and nitrite were computed by multiplying the frequency of intake of each food item by its nitrate and nitrite concentration and summing across all foods. As described previously (19), nitrate and nitrite concentrations were determined from the literature (20–24). Sex-specific portion sizes were derived from the Second National Health and Nutrition Examination Survey (25). Cases and controls with more than five missing food items were excluded from dietary analyses. Among those remaining, intakes for missing foods were imputed using the sex-specific median value among controls.

Statistical analyses

Maximum likelihood estimates of the odds ratios were calculated using unconditional multiple logistic regression analysis to estimate the association between the selected exposure indices and pancreatic cancer. Results were similar for men and women, so only combined results are presented. Risk estimates were adjusted for age, gender, and cigarette use (ever smoked for 6 or more months vs. never). Adjustment for a more detailed smoking variable did not significantly alter results. Therefore, only odds ratios adjusted for the two-level variables are presented. The dietary odds ratios were adjusted for total caloric intake by including it as a continuous variable in the logistic models. For the major analyses, we evaluated whether excluding proxies who were not the spouses changed our findings because other respondents may be less knowledgeable about the subject. Trend tests for categorized exposure variables were performed by assigning ordinal scores to the categories and testing for a non-zero slope. Statistical analyses were performed using SAS version 8.1 software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Approximately 50 percent of cases and 49 percent of controls were excluded because of insufficient drinking water nitrate data. The primary reason for exclusion was due to extensive use of a private well as a drinking water source after 1960. Table 1 shows the characteristics of the cases and controls included and excluded from the community water supplies' nitrate analysis. The median age was similar for both included and excluded cases and controls. As expected, cases and controls included in the community water supplies' nitrate analysis had used community water supplies substantially longer than excluded cases and controls, and private well use was less common. Bottled water use was uncommon in both groups. Compared with the overall study population, the community water supplies' nitrate analysis population included a greater proportion of smokers and a greater proportion with more years of formal education. The proportion of cases and controls in the community water supplies' analysis with a history of pancreatitis and a family history of cancer was similar to the overall study population. As was observed in the overall study population, smoking, pancreatitis, and a family history of cancer were associated with an increased risk of pancreatic cancer.

We divided the study population into quartiles based on the distribution of the controls' community water supplies' average nitrate levels (median, 1.3 (interquartile range, 0.6–2.8) mg of nitrate nitrogen per liter). We observed no associ-

TABLE 1. Distribution of selected characteristics among individuals included in the community water supply nitrate analysis, Iowa, 1985–1987

	Included in analysis*				Excluded from analysis			
	Cases (n = 189)		Controls (n = 1,244)		Cases (n = 187)		Controls (n = 1,190)	
	Mean	SD†	Mean	SD	Mean	SD	Mean	SD
Community water supply in Iowa (years)	48.7	15.5	48.8	15.2	18.8	21.1	17.4	19.6
Private wells in Iowa (years)	13.5	14.5	13.7	14.2	40.3	26.6	45.0	24.5
Bottled water (years)	0.06	0.84	0.21	2.1	0		0.08	0.72
	Cases		Controls		Cases		Controls	
	No.	%	No.	%	No.	%	No.	%
Gender								
Male	102	54.0	775	62.3	100	53.5	826	69.4
Female	87	46.0	469	37.7	87	46.5	364	30.6
Age (years)								
40–54	11	5.8	120	9.7	19	10.2	122	10.3
55–64	47	24.9	290	23.3	45	24.1	258	21.7
65–74	74	39.2	469	37.7	62	33.2	436	36.6
75–85	57	30.2	365	29.3	61	32.6	374	31.4
Education‡								
Less than high school	55	29.7	377	30.5	74	40.2	452	38.2
High school	76	41.1	465	37.7	67	36.4	457	38.6
Greater than high school	54	29.2	393	31.8	43	23.4	275	23.2
Marital status‡								
Married (or living as married)	130	68.8	921	74.1	127	67.9	928	78.05
Not married	59	31.2	322	25.9	60	32.1	261	21.95
Cigarette smoking§								
Never smoker	62	32.8	531	42.7	74	39.6	576	48.4
Former smoker	60	31.8	437	35.1	56	29.9	425	35.7
Current smoker	67	35.5	276	22.2	57	30.5	189	15.9
Pancreatitis‡								
No	137	84.1	1,140	99.1	126	88.1	1,085	98.9
Yes	26	15.9	10	0.9	17	11.9	12	1.1
Cancer in the family‡								
No	66	39.5	635	53.6	76	44.4	560	49.4
Yes	101	60.5	550	46.4	95	55.6	573	50.6
Pancreatic cancer in family‡								
No	158	94.6	1,164	98.2	158	92.4	1,110	98.0
Yes	9	5.4	21	1.8	13	7.6	23	2.0

* Individuals with 70% or greater of their person-years (after 1960) using a community water supply with a known nitrate estimate.

† SD, standard deviation.

‡ Numbers do not sum to total because of missing information.

§ Individuals were categorized as former smokers if they quit smoking more than 2 years before.

ation between pancreatic cancer risk and increasing levels of nitrate in public drinking water supplies (p_{trend} = 0.28) (table 2). Lagging the exposure by 10 years did not substantially change the results (data not shown).

Overall, cases reported a slightly higher total tap water intake of 2.5 (standard deviation, 1.29) liters/day than did controls, whose total tap water intake was 2.3 (standard deviation, 1.13) liters/day (p value = 0.07). Total tap water intake was multiplied by the community water supplies' average nitrate level to obtain the average nitrate consumption from drinking water. No association was found between pancreatic cancer and increasing quartiles of nitrate consumption

TABLE 2. Odds ratios and 95% confidence intervals for pancreatic cancer, by average nitrate level in drinking water from 1960 onward, Iowa*

Average nitrate (nitrate nitrogen, mg/liter)	Cases (n = 189)	Controls (n = 1,244)	Odds ratio†	95% CI‡
<0.6	50	311	1.0	
0.6–<1.3	62	311	1.2	0.79, 1.8
1.3–2.8	28	311	0.54	0.33, 0.89
>2.8	49	311	0.99	0.64, 1.5

* Analysis included only those individuals with 70% or greater (after 1960) person-years using a community water supply with known nitrate estimates.

† Odds ratios adjusted for age, gender, and cigarette use.

‡ CI, confidence interval.

TABLE 3. Odds ratios and 95% confidence intervals for pancreatic cancer by number of years with a community water supply nitrate level at or above 7.5 and 10 mg of nitrate nitrogen per liter after 1934, Iowa*

Years of nitrate nitrogen at or above level	Cases (n = 189)	Controls (n = 1,244)	Odds ratio†	95% CI‡
7.5 mg/liter				
0	140	952	1.0	
1–4	19	119	1.1	0.66, 1.9
>4	30	173	1.2	0.79, 1.9
10 mg/liter				
0	144	1,007	1.0	
1–2	42	199	1.5	1.0, 2.2
>2	3	38	0.58	0.18, 1.9

* Analysis included only those individuals with 70% or greater (after 1960) person-years using a community water supply with a known nitrate estimate.

† Odds ratios adjusted for age, gender, and cigarette use.

‡ CI, confidence interval.

from drinking water. Likewise, when we stratified the average nitrate analysis by the median tap water intake in the controls (2.1 liters/day), we found no association between pancreatic cancer and the nitrate level among those with both high and low tap water intakes (data not shown).

We evaluated the association of drinking water nitrate levels and pancreatic cancer stratified by dietary vitamin C intake and smoking status, factors known to affect endogenous nitrosation. Results were similar among those with vitamin C intake below and at or above the median and among never smokers and current or past smokers (data not shown).

Few individuals in this population were ever exposed to community water supplies' nitrate levels at or above 7.5 or 10 mg of nitrate nitrogen per liter. When exposure at these higher levels did occur, it was usually for only a few years (median years of ≥10 mg/liter, 2; interquartile range, 1–2). We found no significant association between pancreatic cancer risk and years of exposure to community water supplies at or above these levels (table 3). Those with 1–2 years of exposure at 10 mg/liter or more had a 50 percent elevated risk of borderline significance. However, the risk among subjects exposed for more than 2 years at this level was not elevated (odds ratio (OR) = 0.6, 95 percent confidence interval (CI): 0.2, 1.9).

We conducted analyses including only individuals with 90 percent or greater years of nitrate measurements after 1960 (132 cases, 870 controls) to further reduce possible misclassification by unknown nitrate levels. The results were not substantially different from those presented here, although the odds ratio for the highest quartile of average nitrate was somewhat lower (OR = 0.7, 95 percent CI: 0.4, 1.2). We also evaluated whether the risk estimates changed if we excluded those who requested and completed proxy questionnaires but for whom the respondent was not the spouse and was therefore less likely to share the same residential and water source history (68 cases). Our results were similar to those reported here.

We evaluated the association between pancreatic cancer risk and total years of using a private well as a drinking water source. Shallow wells in Iowa tend to have higher nitrate levels than do deep wells (26), so we also evaluated the association with years of shallow (<50 feet or 15.24 meters) well use. We found no association between pancreatic cancer risk and years of well use overall or years using a shallow well. The odds ratio for shallow well use greater than 10 years was 0.6 (95 percent CI: 0.4, 1.0) (table 4).

Dietary intake of nitrate showed a different association with risk among men and women (table 5). Among men, there was no association with nitrate intake from foods; however, among women, the risk decreased with increasing quartiles of intake (p_{trend} = 0.04). The inverse association among women was not found when we limited the analysis to self-respondents and proxies who were the husbands of the subject. Higher intake of dietary nitrite was associated with a modest increased risk of pancreatic cancer in both men and women (table 5). We evaluated animal and plant

TABLE 4. Odds ratios and 95% confidence intervals for pancreatic cancer cases and controls by years of shallow (<50 feet*) private well use, Iowa, 1985–1987†

Years	Cases (n = 189)	Controls (n = 1,244)	Odds ratio‡	95% CI§
0	162	959	1.0	
1–10	8	91	0.57	0.27, 1.2
>10	19	194	0.63	0.38, 1.0

* Metric equivalent: 15.24 meters.

† Analysis included only those individuals with 70% or greater known person-years from 1960 onward using a community water supply with nitrate estimates.

‡ Odds ratios adjusted for age, gender, and cigarette use.

§ CI, confidence interval.

TABLE 5. Odds ratios and 95% confidence intervals for pancreatic cancer cases and controls by quartiles of consumption of nitrate and nitrite from the diet, Iowa, 1985–1987*

Men					Women				
	Cases (n = 141)	Controls (n = 1,247)	Odds ratios†	95% CI‡		Cases (n = 122)	Controls (n = 639)	Odds ratios†	95% CI
Dietary nitrate (mg/day)					Dietary nitrate (mg/day)				
<58	26	298	1.0		<63	39	164	1.0	
58–82	33	311	1.1	0.63, 1.9	63–90	33	157	0.99	0.58, 1.7
83–117	39	311	1.2	0.70, 2.0	91–126	24	158	0.64	0.36, 1.1
>117	43	327	1.0	0.60, 1.8	>126	26	160	0.53	0.29, 0.97
Dietary nitrite (mg/day)					Dietary nitrite (mg/day)				
<0.75	15	233	1.0		<0.56	18	144	1.0	
0.75–0.98	22	307	1.0	0.52, 2.0	0.56–0.71	32	146	1.8	0.94, 3.4
0.99–1.30	40	333	1.5	0.81, 2.9	0.72–0.93	32	168	1.4	0.72, 2.6
>1.30	64	374	1.5	0.79, 3.0	>0.93	40	181	1.3	0.65, 2.5
Dietary nitrite from animal sources (mg/day)					Dietary nitrite from animal sources (mg/day)				
<0.22	9	264	1.0		<0.13	13	148	1.0	
0.22–0.31	22	282	2.1	0.95, 4.8	0.13–0.18	32	164	2.4	1.2, 4.7
0.32–0.53	60	359	3.8	1.8, 8.0	0.19–0.26	26	147	1.9	0.94, 4.0
>0.53	50	342	2.3	1.1, 5.1	>0.26	51	180	3.2	1.6, 6.4

* Analysis included only those with an adequate dietary history (less than six missing foods).
† Odds ratios adjusted for age, cigarette use, and caloric intake.
‡ CI, confidence interval.

sources of nitrite separately (table 5). Among men and women, we found a significant positive association between higher animal nitrite intake and pancreatic cancer (p_{trend} = 0.02 and <0.01 for men and women, respectively). Odds ratios were similar after limiting the analysis to only self-respondents and proxies who were spouses of the subject. Plant sources of nitrite were not significantly associated with risk among men and women before or after exclusion of nonproxy respondents.

DISCUSSION

The average nitrate level in community water supplies over an approximately 25-year period was not associated with pancreatic cancer risk in this study population. Average exposure levels were relatively low. For the large majority (75 percent) of individuals, less than 10 percent of daily nitrate intake came from drinking water; most was dietary (primarily vegetables). The majority of nitrate intake can come from drinking water when levels approach the maximum contaminant level of 10 mg/liter. However, we estimated that only 1 percent of the study population received approximately 50 percent of their daily nitrate intake from water. Those with 1–2 years of exposure at or above the maximum contaminant level of nitrate nitrogen (10 mg/liter) had a 50 percent elevated risk that was of borderline significance; however, the risk was not elevated for exposure for a longer period at this level. Our analysis of exposure at or above the maximum contaminant level was limited because of the small number of subjects who had experienced any exposure at this level. Controlling for known pancreatic cancer risk factors and potential nitrosation modulators did not alter our drinking water nitrate findings.

Dietary nitrate, mostly of vegetable origin, was not associated with an increased risk of pancreatic cancer. Among women but not men, increasing intake was associated with decreasing risk. In contrast, higher intakes of dietary nitrite derived from animal sources were associated with significantly increased risks of pancreatic cancer among both men and women.

Measurements of nitrate levels from private wells were not available for this study; consequently, individuals with the highest potential nitrate exposures from drinking water were not included in the analysis. We did not find an association between pancreatic cancer risk and years of private well use overall or years of shallow well use. Well depth is an important predictive factor for nitrate levels in Iowa and other areas of the United States (26, 27). However, other factors are also important determinants of nitrate levels in private wells including well construction, location with respect to nitrogen sources such as agricultural fields, septic tanks and animal feedlots, and characteristics of the aquifer in which the well is located (28, 29). Lack of information about these factors likely resulted in substantial misclassification of nitrate exposure in our analysis of duration of private well exposure.

Our results for pancreatic cancer risk with average nitrate level in community water supplies are consistent with results of a recent cohort study of older women in Iowa (16). Weyer et al. (16) evaluated pancreatic cancer risk by quartile cutpoints of the average nitrate level in 1955–1988 for a women's Iowa community water supply at the time of enrollment. Quartile cutpoints of nitrate nitrogen were very similar to those in our study (<0.36, 0.36–1.00, 1.01–2.46, >2.46 mg/liter); increasing intake was not associated with risk (ORs were 1.0, 0.77, 1.20, and 0.65, respectively). There was potential for some overlap between the women in our study and in the study by Weyer et al. (16). Female cases diagnosed with pancreatic cancer after the cohort study's baseline survey in 1986 and during 1987 were eligible to be included in both studies. However, the overlap in cases was small because the prospective cohort study enrolled only 43 percent of eligible older women, and incident pancreatic cancer cases in that study were ascertained for an additional 10 years (1988–1998).

A few studies have evaluated dietary intakes of nitrate and nitrite and pancreatic cancer risk, although none evaluated animal and vegetable products separately. A case-control study in 1990 by Howe et al. (30) found no association between dietary intake of nitrate and nitrite and pancreatic cancer risk. A case-control study in 1991 by Baghurst et al. (31) reported a significantly decreased risk associated with the highest quartile of dietary nitrate intake; there was no association with dietary nitrite intake. Most studies have reported elevated risks associated with increased consumption of smoked or processed meats (32). Processed meat intake, specifically a group consisting of bacon and smoked ham, was associated with increased risk of pancreatic cancer in a case-control study conducted in Sweden (33). A case-control study using only direct interviews did not find an association between pancreatic cancer risk and the consumption of processed meat in either men or women (34). Pork products (including processed and unprocessed) were associated with an increased risk of pancreatic cancer in Louisiana (35). These findings were strongest for individuals with Cajun ancestry and may be related to preparation methods.

It is difficult to separate exposure to nitrite from other aspects of preserved meat consumption, including the cooking method. Preparation methods such as frying and grilling have been associated with an increased risk of pancreatic cancer (33). Future studies that use a more detailed dietary assessment tool, including questions on food preparation, will be more informative.

Our findings demonstrate that it is important to consider the sources of the dietary nitrate and nitrite in the evaluation of pancreatic cancer risk. Nitrate is derived almost entirely from vegetables that are known to contain inhibitors of in vivo nitrosation, which may in part explain their consistent inverse associations with many epithelial cancers (12, 14). Therefore, nitrate and nitrite consumed in vegetable products are not likely to result in significant formation of *N*-nitroso compounds. Animal products containing nitrite (primarily processed meats) are a source of amines and amides, which are also precursors of *N*-nitroso compounds. Furthermore, a number of preformed *N*-nitroso compounds are found in processed meats (36, 37). As a result, consumption of nitrite from animal products should result in more substantial exposure to *N*-nitroso compounds than plant-based products.

Controlled human studies have demonstrated increased endogenous nitrosation when subjects are given oral doses of proline, an amino acid, and then ingest drinking water with elevated nitrate levels, indicating the potential for the formation of carcinogenic *N*-nitroso compounds (10, 13). In these studies, the elevated nitrate levels were higher than the average nitrate levels for the large majority of our study population. It is not known if substantial nitrosation occurs at lower levels.

The proposed pathway linking nitrate to cancer is complex. Individual variation in the rate of nitrosation results in various levels of exposure to *N*-nitroso compounds. Differences between individuals have not been totally explained; however, some factors have been identified. Smoking has been identified as a possible nitrosation enhancer (38, 39). Smokers were found to have significantly higher rates of *N*-nitrosoproline production after consuming nitrate and proline than their nonsmoking counterparts. This could indicate that smokers are at an elevated risk of exposure to carcinogenic compounds when consuming nitrate in their drinking water. Vitamin C is a well-documented inhibitor of nitrosation (14). Individuals consuming low levels of vitamin C may also be at an increased risk. We evaluated these two possible nitrosation modulators and found no evidence of interaction.

The strengths of our study include the high response rates among cases and controls, information on lifetime water sources and historical nitrate levels for Iowa public water supplies, and our ability to evaluate effect modification by vitamin C intake and smoking. Our study did not have sufficient power to evaluate risk at nitrate levels above the maximum contaminant level because of the lack of exposure data for private wells and infrequent high exposure among public water supply users. Studies of exposure to disinfection by-products and cancer risk have often seen the greatest elevated risks only after 30 or more years of exposure (15, 17, 18). Our exposure period of 27 years would have been insufficient if a similar induction period occurs for nitrate.

Our drinking water nitrate analyses were limited to those who used Iowa public supplies with nitrate estimates for 70 percent of their person-years from 1960 onward. By not including study subjects with a high proportion of years using a private well, we limited our analysis to nitrate levels that were largely below an average level of nitrate nitrogen (5 mg/liter). This exclusion was necessary to limit misclassification due to unknown but likely higher nitrate levels in private wells. A similar proportion of cases and controls were excluded from the public supply analyses, and the mean years of private well use were similar between cases and controls. Thus, it is unlikely that this exclusion would bias the results of the public supply nitrate analyses. Misclassification of drinking water nitrate exposure may have occurred as a result of infrequent monitoring in the early years of the study. However, cases and controls had similar numbers of measurements for each decade (19); therefore, misclassification would likely be nondifferential and would be expected to bias odds ratios toward the null.

A limitation of this study was the large number of surrogate respondents among cases. Although the median time between diagnosis and interview was only 1 year, due to the severe morbidity associated with pancreatic cancer, only about 10 percent of cases completed the questionnaire themselves, whereas about 85 percent of controls were self-respondents. Thus, there was the potential for differential misclassification of exposure due to the likely poorer quality of the information among cases. We evaluated this possible bias by excluding proxy respondents who were not the spouse. Spouses are likely to have shared a substantial portion of their residence history with the subject and would therefore be expected to accurately report residential and dietary information, whereas other proxy respondents may be less knowledgeable. Results for the drinking water analysis did not differ substantially from those found using all proxy respondents. Likewise, excluding proxies who were not spouses did not alter results for the dietary nitrate and nitrite analysis in men substantially; however, among women, the odds ratios for dietary nitrate and nitrite were attenuated.

In summary, we did not find evidence for an association between drinking water nitrate levels below the maximum contaminant level and pancreatic cancer. However, this hypothesis deserves further evaluation in future studies among populations with higher drinking water nitrate exposure. Our results suggest a role for nitrite derived from animal sources as a pancreatic cancer risk factor. Further research with a detailed analysis of dietary nitrate and nitrite is needed to more precisely define the relation between *N*-nitroso compounds and pancreatic cancer.

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